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Diaporthe henanensis sp. nov., an endophytic fungus in *Ziziphus jujuba* from China

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Abstract—A species of *Diaporthe* isolated from fruit of *Ziziphus jujuba* during a study of the diversity of endophytic fungi in Zhengzhou, Henan, China, was found to be morphologically and molecularly distinct from known species. This new species, proposed as *Diaporthe henanensis*, differed from other *Diaporthe* species by its pyriform, relatively wide γ-conidia. Analysis of multi-locus DNA sequences (ITS, TUB, HIS) showed that *D. henanensis* was closely related to *D. nobilis, D. neilliae*, and *D. alnea*.

Key words—endophytes, *Phomopsis*, *Ascomycota*, systematics

Introduction

Diaporthe (≡ *Phomopsis*) species are common plant pathogens, endophytes or saprobes (Webber & Gibbs 1984, Carroll 1986, Boddy & Griffith 1989, Rehner & Uecker 1994, Udayanga et al. 2011). In recent years there has been an acceleration in the discovery and naming of new *Diaporthe* species recognized primarily through molecular analyses (Santos & Phillips 2009; Santos et al. 2011; Crous et al. 2011; Udayanga et al. 2012, 2014).

Udayanga et al. (2011) suggested *Diaporthe* species should be identified by a combination of molecular, morphological, cultural, pathological and mating type data. Tan et al. (2013) reported six new *Diaporthe* species from Australia through ITS, TUB, and TEF gene sequence analyses. Huang et al. (2013) reported two *Diaporthe* species based on ITS, EF, TUB, and CAL gene

[#] These authors contributed equally to this study.

Table 1 *Diaporthe* species analysed in this study. Newly deposited sequences are in bold.

Species	Strain	Host	Locality	GenBank accession numbers		
				ITS	TUB	HIS
D. alleghaniensis	CBS495.72	*Betula alleghaniensis*	Canada	KC343007	KC343975	KC343491
D. alnea	CBS146.46	*Alnus* sp.	—	KC343008	KC343976	KC343492
	CBS159.47	*Alnus* sp.	—	KC343009	KC343977	KC343493
D. eres	CBS375.61	*Malus sylvestris*	—	KC343088	KC344056	KC343572
	CBS445.62	*Alliaria officinalis*	Netherlands	KC343091	KC344059	KC343575
D. gardeniae	CBS288.56	*Gardenia florida*	Italy	KC343113	KC344081	KC343597
D. henanensis	HHAUF12291	*Ziziphus jujuba*	China	**KC898258**	**KF600608**	**KF600609**
	HHAUF12292	*Ziziphus jujuba*	China	**KC898259**	**KF600610**	**KF600611**
D. juglandina	CBS121004	*Juglans* sp.	USA	KC343134	KC344102	KC343616
D. neilliae	CBS144.27	*Spiraea* sp.	—	KC343144	KC344112	KC343628
D. nobilis	CBS116953	*Pyrus pyrifolia*	New Zealand	KC343147	KC344115	KC343631
	CBS333.89	*Hedera helix*	Yugoslavia	KC343152	KC344120	KC343636
D. vaccinii	CBS122115	*Vaccinium corymbosum*	USA	KC343226	KC344194	KC343710
	CBS122114	*Vaccinium corymbosum*	USA	KC343225	KC344193	KC343709

sequence analyses. Gomes et al. (2013) investigated 243 isolates of *Diaporthe*, divided them into 95 species using multi-locus DNA sequence data (incl. ITS, HIS, TUB, CAL, TEF1), and concluded that future species descriptions should include at least ITS as well as HIS or TUB sequence data.

Ziziphus jujuba Mill. (*Rhamnaceae*) is a small deciduous tree grown in China for its edible fruit. During a study of the diversity of endophytic fungi in Zhengzhou, Henan, China, a fungus regularly isolated from fruit of *Z. jujuba* was shown to represent a novel species of *Diaporthe*, which is described here as *Diaporthe henanensis*.

Materials & methods

Isolates, media, and culturing

Healthy fruit of *Ziziphus jujuba* was collected near Zhengzhou, Henan Province, China. Tissue pieces of about 3 mm^3 were treated with 75% ethanol for 30 s, surface disinfected in 3% sodium hypochlorite for 3 min, rinsed with sterile distilled water, then placed on potato carrot agar (PCA) plates at 25 °C for one week. Young colonies were transferred to potato dextrose agar (PDA) plates and kept at room temperature under alternating light and dark periods (Xie et al. 2010) for conidium production. Morphological characteristics of conidia and conidiophores were microscopically observed after 10 d.

The type specimen was deposited in the Herbarium Mycologicum Academae Sinicae, Beijing, China (HMAS), and cultures were deposited in China General Microbiological Culture Collection Centre, Beijing, China (CGMCC) and in the Herbarium Henan Agricultural University: Fungi, Zhengzhou, China (HHAUF).

DNA extraction, polymerase chain reaction, and sequencing

Total DNA was extracted from mycelium scraped from the surface of a PDA plate with the Cell Genomic DNA Isolation Kit (LifeFeng, Shanghai, China) following the manufacturer's instructions. The primers ITS1 and ITS4 (White et al. 1990) were used to amplify the ITS1-5.8S-ITS2 rDNA region (ITS) (Luo & Mitchell 2002). The primers T1 (O'Donnell & Cigelnik
1997) and bt2b (Glass & Donaldson 1995) were used to amplify part of the tubulin gene (TUB). The primers CYLH3F (Crous et al. 2004) and H3-1b (Glass & Donaldson 1995) were used to amplify part of the histone H3 gene (HIS). PCR was carried out using an Eppendorf PCR thermal cycler (Germany). The reaction products were analyzed by electrophoresis on a 1.0% agarose gel in tris-acetate-EDTA (TAE) buffer with ethidium bromide (0.1 mg/mL) and visualized under UV light (Xie et al. 2010). The PCR products were sequenced by Shanghai Sangon Biological Engineering Technology & Service Co., Ltd., China.

The ITS sequences were initially aligned with published ITS sequence data of *Diaporthe* spp. obtained from the NCBI using ClustalW in MEGA ver. 5.2 (Tamura et al. 2011). *Diaporthella corylina* was selected as the outgroup taxon. A neighbor–joining (NJ)

analysis using the Kimura-2 parameter with Gamma distribution was applied to nine similar species and the closest phylogenetic neighbours were selected for a combined analysis using ITS, TUB and HIS genes (Table 1). A Maximum likelihood tree using the combined dataset was generated in MEGA 5.2 using the Tamura-Nei substitution model with Gamma distribution. Bootstrap support values with 1000 replications were calculated for tree branches (Tan et al. 2013).

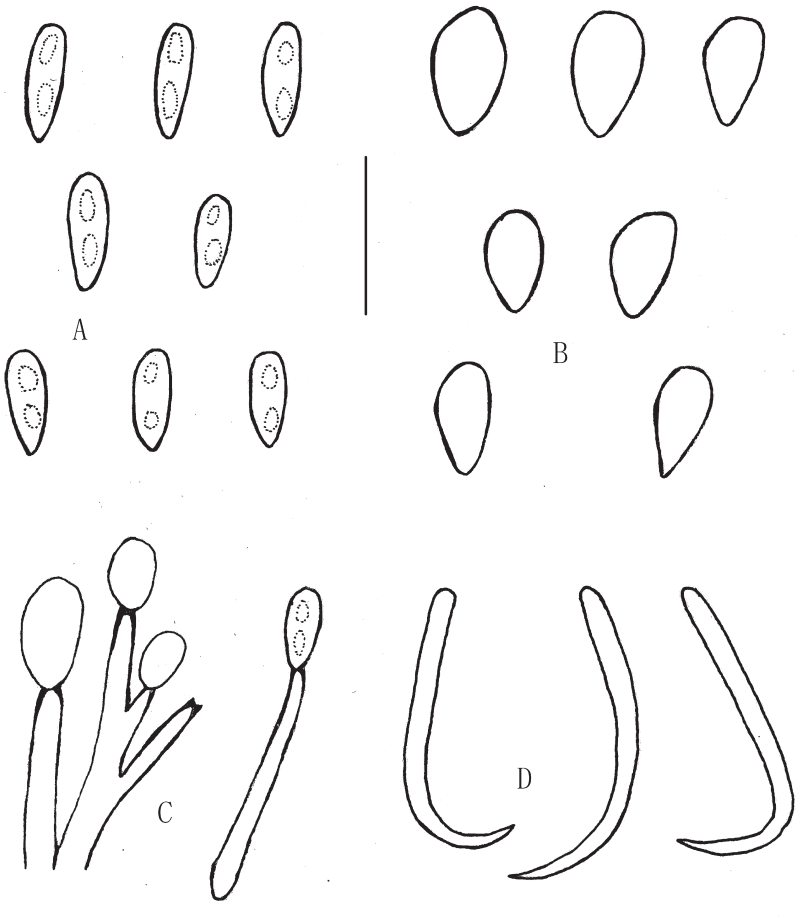

Fig. 1 *Diaporthe henanensis* on PDA.
A. α-conidia; B. γ-conidia; C. Conidiophores; D. β-conidia.
Scale bar = 10 μm.

Taxonomy

Diaporthe henanensis Yi Yang, H.Y. Wu & Meng Zhang, **sp. nov.** Fig.1

MycoBank MB 804356

Differs from all other *Diaporthe* spp. by its γ-conidia that are broadly pyriform and by its multi-locus gene sequence data.

Type: China, Henan Province, Zhengzhou, from healthy fruit of *Ziziphus jujuba*, 26 Sep. 2012, coll. Y. Yang (**Holotype**, HMAS246233 [dried culture]; ex-type strain, CGMCC3.17639 = HHAUF12291; GenBank KC898258, KF600608, KF600609).

Etymology: Named for the collection locality.

Pycnidia scattered in PDA, dark brown to black, globose to subglobose, 800–1700 μm diam. Conidiophores subcylindrical, hyaline, smooth, simple or branched, 14–18 × 1.5–2.0 μm. Conidiogenous cells phialidic, cylindrical, tapering towards the apex, with periclinal thickening. Collarettes inconspicuous. Three kinds of conidia were produced: α-conidia, unicellular, hyaline elliptical or clavate, one end obtuse, the other acute, 2-guttulate, 5–8 × 2–3 μm; β-conidia hyaline, aseptate, without guttules, filiform, curved or hamate, with obtuse ends, 15.5–31 × 0.8–1.5 μm; γ-conidia, pyriform, 5–7 × 3.5–5.0 μm.

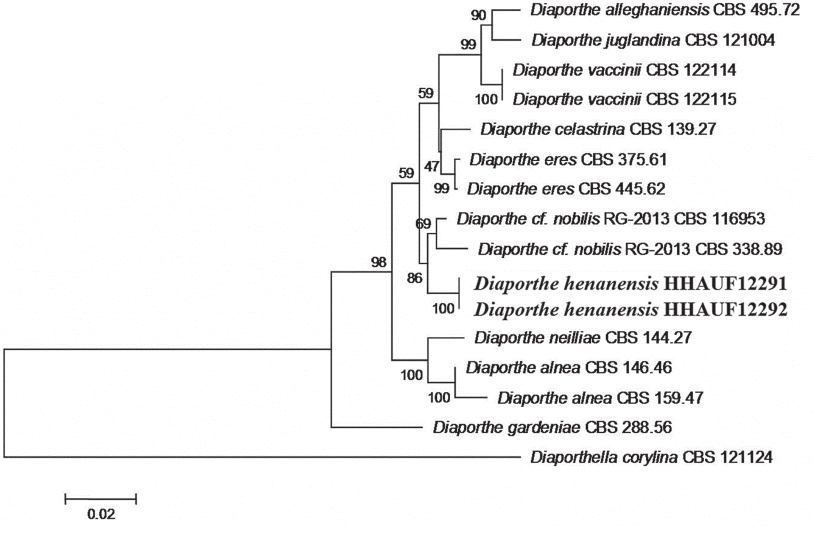

Fig. 2 Maximum likelihood tree inferred from combined analysis of three genes (ITS, TUB, HIS). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree was rooted to *Diaporthella corylina*. The newly sequenced strains of our new species are in bold..

Additional material examined: **CHINA**, **Henan Province**, Zhengzhou, from healthy fruit of *Ziziphus jujuba*, 26 Sep. 2012, coll. Y. Yang (HHAUF12292; GenBank KC898259, KF600610, KF600611).

Discussion

All other *Diaporthe* species differ from *D. henanensis* either by lacking γ-conidia or by producing γ-conidia that are not broadly pyriform (Crous et al. 2012, Gomes 2013, Uecker 1988, Qi et al. 2007). The initial ITS sequence data support *D. nobilis*, *D. neilliae*, *D. alnea*, *D. juglandina*, *D. eres*, *D. gardeniae*, *D. alleghaniensis*, and *D. vaccinii* as the most closely related phylogenetic neighbours. The two isolates clustered with each other in a single clade with 100% bootstrap support in the ML tree inferred from analysis of three genes (ITS, TUB, HIS; Fig. 2).

Many species of *Diaporthe* are known to be endophytic on a range of host plants (Murali et al. 2006, Chaeprasert et al. 2010, Rocha et al. 2011, Sun et al. 2011). In this study, *D. henanensis* was isolated and described as new based on its distinctive morphology (broadly pyriform γ-conidia) and multi-locus gene sequence data (ITS, TUB, HIS).

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